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ARCHITECTURE OF DERMATOPHYTE CELL WALLS:
ELECTRON MICROSCOPIC AND BIOCHEMICAL ANALYSIS

Yasuo Kitajima and Yoshinori Nozawa

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ARCHITECTURE OF DERMATOPHYTE CELL WALLS: ELECTRON MICROSCOPIC
AND BIOCHEMICAL ANALYSIS

Yasuo Kitajima and Yoshinori Nozawa*

INTRODUCTION

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The cell walls of true fungi not only physically protect the structure and function of the cells from the outside environment, but also have biological functions and properties with regard to the presence of specific antigens, the strength of pathogens, and dimorphism of the fungus. Consequently, clarification of the structure and functions of these complex cell walls is said to be one effective approach in explaining therapeutic problems with regard to current mycoses. A molecular structural model of cell walls has been seen in the field of bacteriology in biochemical and morphological research, particularly in research using electron microscopes. Various antibodies have been developed and their mechanisms have been clarified with this extensive research [1-3]. The importance of research on cell structures, including cell walls, is noted. On the other hand, research on the cell walls of true fungi has been considerably delayed in comparison to that of bacteria. Nevertheless, recent interest has been noted with regard to the cell walls of pathogenic true fungi and important information on dimorphism [4-5] and pathogenicity [16-22] is being accumulated.

Morphologically, the cell walls of true fungi are constructed from a mesh-like structure made of microfibriles and an amorphous matrix, in which there are crevices between the fibriles

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(amorphous matrix). These two structures appear to give the cell walls a layered structure. Moreover, chemically, the cell walls are made from polysaccharides, such as mannan, various glucans, etc., and chitins containing small amounts of peptides [23]. At the heart of these biochemical analyses are the summaries of taxonomical and morphological data of Bartnicki-Garcia [24]. There have recently been structural models of the cell walls of dermatophytes [25] and scientific progress in this field is anticipated in the future.

Here we will attempt to clarify the structure of cell walls of dermatophytes by the following items, centering on research results on the cell walls of Trichophyton mentagrophytes [26, 28-30] and Epidermophyton floccosum [27, 31-33], which have been studied for the past 10 years in our laboratories.

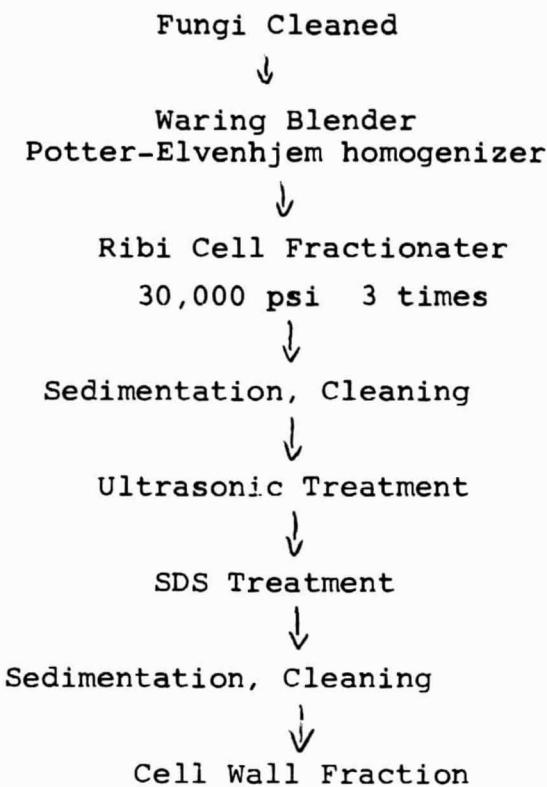
1. Separation and preparation of cell walls
2. Microstructure of cell walls by electron microscopy
3. Chemical composition of cell walls
 - 1) sugar component
 - 2) protein component
 - 3) fat component
4. Structural model of cell walls
 - 1) hypha cell walls
 - 2) septate cell walls
5. Morphological structure of cell walls

1. Separation and Preparation of Cell Walls

The first requirement in the study of cell walls is to prepare cell wall fractions that are of a high purity and are as similar as possible to the native. One reason for the considerable delay in the study of cell walls of dermatophytes in comparison to that of enzyme-type true fungi is that these cell walls are usually very difficult to prepare. Moreover, preparation is further complicated by the fact that it is difficult to completely decompose the cells because the fungi have many septae. Consequently, in order to remove the cells that have not been decomposed because of the septae, preparation must be carried out by density gradient-type centrifugation [29]. Moreover, cell wall fractions with the necessary purity are not obtained and therefore, the entire cell must first be completely destroyed in order to separate cell wall fractions of a high purity.

Tests on various methods have been reported with regard to decomposition of true fungi cells. For instance, ultrasonic treatment, Sorvall omnimixer [35-37], French press 38-39, Braun homogenizers [40-42], Waring blenders [42], or combinations of these have been used. Although results that are just as good as those seen with the Braun homogenizer are obtained [26], the Ribi cell fractionator method gives the most efficient results [29,21]. By means of the Ribi cell fractionator (Ivan Sorvall, Inc., Norwalk, Connecticut, U.S.A.), a high pressure is exerted on the solution containing fungi, which is placed in a copper cylinder (approximately 150 ml). The solution is obtained with a needle valve and cells are destroyed by exposure to a suddenly reduced pressure.

Figure 1 Method for Preparing Cell Walls of Dermatophytes [31]



The most efficient method for preparation of fungus cell walls is shown in the figure (Figure 1). First of all, the cleaned fungi are sufficiently treated with a Waring blender to obtain a fungi solution. Furthermore, a uniform solution is then obtained by homogenization with the glass Potter-Elvehjem homogenizer. The fungi that have been thus treated can then be easily passed through the needle valve of the Ribi cell fractionater. The efficiency of fungi destruction is thereby improved. Next, decomposition of the cells in a Ribi cell fractionater is performed three times under a high pressure of 30,000 pounds/inch³. The dermatophytes of Trichophyton, Epidermophyton, and Microsporum are all destroyed. After these products have been rinsed several times, the membrane component that adheres to the inside of the cell walls is removed by ultrasonic treatment. The final solution is incubated for 1 hour at room temperature in 1% SDS (sodium dodecyl sulfate) and is then rinsed and separated. According to electron microscopic studies, the cell wall fraction of E. Floccosum prepared in this way is very pure and 280 nm absorption by proteins in the supernatant liquid is not noted (Figure 2a, b, c). This method was also used by Barran 44 for Fusarium sulphureum and it has recently

been reported that good results were obtained. In addition, the protein decomposing enzyme method [29,45], the RNase method [29], and the density gradient centrifugation method [29] have been reported. Although there are not problems with cell wall purity, these methods do have disadvantages from the points of the yield rate, operation complexity, etc.

Therefore, with regard to mechanical fractionation as seen with the Ribi cell fractionater, the difference in resistivity between different types of fungi is considerable, even when the same dermatophytes are used, and should be noted. For instance, in contrast to the fact that all cells were fractionated by treatment of *E. Floccosum* one time under 30,000 pounds/inch², the same extent of fractionation of *T. mentagrophytes* is seen only after 3 treatments. This fact shows that there is a difference in the structure of cell walls.

Next, the purity of the cell wall fractions differed with the cells, nucleus, and other membrane systems. In many reports the results of differential phase microscopes [45, 46] and of methylene blue staining tests [5, 47, 48] are cited. Although optical microscopes are sufficient for determining the presence of mixtures of unfractionated cells, it is also necessary to use the results of electron microscopic studies, that is, thin film methods, shadowing methods, and scanning electron microscopic methods to determine whether there are mixtures of cell substances. On the other hand, there are also methods that determine the amount of proteins [49,50] and nucleic acids [49,51] by absorption of the supernatant liquid at 280 nm and 260 nm. In addition, lymph fat substances can also be determined [31]. In any case, it appears that both electron microscopic methods and biochemical methods are essential for the determination of the purity of the cell wall preparation.

2. Microcell Structure by Electronmicroscopic Study of Cell Walls

Thin slice electron microscopy (electron microscopy), shadowing, scanning electron microscopy, and etching microscopy are widely used to observe the microstructure of cell walls of fungi.

In general, when the cells of true fungi are observed by thin specimen microscopy, a layered structure with a thickness of

approximately 0.1-0.5 is obtained. Dermatophytes appear to have the same structure and reports have been seen on Trichophyton [52-54] Epidermophyton [55-56], and Microsporum [57-58]. When the cell walls of E. Floccosum were observed after being fixed with potassium permanganate and stained with uranium sulfate, it was noted that the outer layer of the cell walls was very thin at 0.01-0.05 μ . There was also a layer with a low electron density of 0.1-0.2 μ and a layer with a somewhat higher electron density of 0.1-0.2 μ was found inside the cell wall (Figure 2b) [31]. The boundary of the two inside layers was not clear. However, it was very obvious that the outside layer was a very thin layer. These findings are in agreement with the results of cell wall observations on untreated cells [55,56]. As a rule, this 3-layered cell structure is also noted in thin specimens of cell walls of Trichophyton [52-54] and Microsporum [57-58].

On the other hand, according to the shadowing method, the cell walls of dermatophytes are also free from microfibriles and an amorphous matrix with crevices. In general, the inside surface is a clear net-like structure because of these microfibriles and the outer surface does not have a clear fibrile structure. Figure 3 shows the shadowing micrographs of the inside surface of E. Floccosum [31]. A net-like structure where the microfibriles are arranged irregularly is seen and an area where fibrile crevices are noted is clear. This structure is also seen in the cell walls of T. rubrum, T. mentagrophytes [20], and M. Coocki. On the other hand, Figure 4a shows the outer surface of the cell walls of E. floccosum. A fibrile structure is not obvious. However, in examples that were SDS treated the structure mainly consists of proteins (mentioned later), as shown in Figure 4b. Therefore, it can be said that a fibrile structure is seen in the bottom layer. These fibers run parallel to the hyphae axis and form bundles. A fibrile structure that differs from that of the inside surface is therefore formed [31].

Thereupon, the nonfibrile structure of the outside surface appears to be the same as the outer surface with a high electron density of the 3-layered structure that was seen with thin specimens [33]. This layer is physiologically different from the fibrile section of the 2nd inner layer when freeze-fracture replicas are used. Figure 5 shows an electron micrograph of the replicas. A relatively smooth surface without fibrile structures is seen. This inside layer has a particle-like structure. The particle structure is reported to

be different depending on the type of fungus that is studied [50]. Furthermore, with regard to freeze fracture of the outside layer, the cell walls of Geotrichum candidum [60], Penicillium urticae[61], etc. is reported to have a "rodlet pattern." The outer layer of these cell walls is structurally different from the inner layer in thin specimen micrographs, shadowing micrographs, and freeze fracture micrographs. This is a significant point from a chemical sense and, as will be mentioned later, we were successful in preparing only the outer layer and explaining its chemistry [33].

As was mentioned above, as a result of morphological studies, it appears that the cell walls of the dermatophytes of T. mentagrophytes and E. floccosum have a 3-layered structure. The outside layer is very thin, but there is a structure where there are no fibers and the electron stainability is very high. The 2nd layer is made from microfibriles and an amorphous matrix.

3. Chemical Composition of Cell Walls

In order to determine the chemical structure of the cell walls of true fungi, it is necessary to separate the high molecular weight components, such as polysaccharides, peptides, etc, which are one structural component. In order to do this, the cells must be decomposed with a strong acid or alkaline water and enzyme treated [51]. Therefore, tests on the properties of the isolated polymers are necessary[51]. We have carried out detailed studies on the hydrolysis and gas chromatography conditions necessary for analysis of the polysaccharides in the cell walls of dermatophytes [29,62].

Chemical analysis of the cell walls of dermatophytes has first been reported by the X-ray diffraction studies of acid and alkali-treated samples prepared by Blank (1953) [63].

Chitin was first discovered. Then Bishop[64], Shah and Knight [36] and the authors of this text [26-33] reported on analytical results of prepared cell walls. However, it cannot be said that any of the results were satisfactory. The chemical composition of the cells walls of T. mentagrophytes, E. floccosum, M. Canis, and M. gypseum are shown in Table 1.

1) Sugar Component

As shown in Table 1, approximately 80% of the cell walls of dermatophytes is sugar, that is, neutral sugar (glucose and mannose) and amino sugars (N-acetylglucosamine).

The first report of neutral sugars in the cell walls of dermatophytes was from studies on T. mentagrophytes and E. fluccosum. However, Cummins and Harris (1968) [65] also detected traces in M. gypseum. Then Bishop (1962) [64] reported that both glucose and mannose were present as components of cell walls of all dermatophytes. However, according to Shah and Knight in 1968, the main component in analyses by paper chromatography was glucose. Only 10% of mannose was detected [36]. Furthermore, in the same report it is mentioned that although traces of galactose were found in M. gypseum, none was detected in the cell walls of T. mentagrophytes [29] and E. floccosum [31]. The amount of galactose in both of these cell wall types is less than 1%. However, the ethanol sediment of soluble fractions that were extracted for 5 hours with 1-N NaOH at 100°C was a polysaccharide, probably galactomannone [30]. Consequently, although only 1% or less of galactose is seen from the cell walls themselves, by isolation, this is noted as a specific high molecular weight compound. Polysaccharides whose main components are galactomannone have been studied as components that have important specific antigen activity from an immunological standpoint [16-19].

Furthermore, the amino sugars contained in the cell walls of dermatophytes are assumed to be chitin because of the fact that glucosamine is eluted by hydrolytic decomposition of the cell walls, the fact that N-acetylglucosamine is eluted from the cell walls with chitinase decomposition, and the fact that by S-ray diffraction of the residue of the cell fraction after acid and alkali decomposition, the same pattern as seen with standard chitin is obtained [29, 21, 36, 63] (figure 6). The sugar compositions vary with the type of fungus and can be used in taxonomical classifications [54].

Table 1 Chemical Composition of Dermatophyte Cell Walls

	<i>T. mentagrophytes</i> ^{*1)}	<i>E. floccosum</i> ^{*2)}	<i>M. canis</i> ^{*3)}	<i>M. gypseum</i> ^{*3)}
a 総中性糖	51.9%	42	48.3	56.6
b グルコース	36.2	35	37.5	45.8
c マンノース	11.7	7	11.4	6.7
d ガラクトース	+	+	-	-
e N-アセチルグルコサミン	30.4	34	26.6	29.7
f 蛋白質	7.8	10	6.8	3.5
g 脂質	6.6	6	4.0	3.3

^{*1)} 文献29^{*2)} 文献31^{*3)} 文献36

*1) reference 29 *2) reference 31 *3) reference 36

Key: a. total neutral sugars

- b. glucose
- c. mannose
- d. galactose
- e. N-acetylglucosamine
- f. protein
- g. fat

2) Protein Component

Although research on proteins contained in dermatophyte cell walls indicates that 7-10% of the cell wall is occupied by protein, analyses are limited to the structure of amino acids in the total cell wall. Shah and Knight [36] have carried out amino acid analyses on cell walls of *T. mentagrophytes*, *M. canis*, *M. gypseum* and *E. floccosum* and have reported that although a large amount of methionine is found in *M. canis* and *M. gypseum*, almost none is seen in *T. mentagrophytes* and that the opposite correlation is noted with regard to cysteine. Moreover, a large amount of leucine is seen in *T. mentagrophytes* and *M. canis*, but little is noted in *E. floccosum* and *M. gypseum*. The same results were obtained in our studies on *T. mentagrophytes* and *E. floccosum* [29, 30]. In general, a large amount of amino acids that appear to participate in bonding with sugars is found in these dermatophytes (glycine, alanine, glutamic acid, aspergilllic acid, seronin, serine, and leucine) [66].

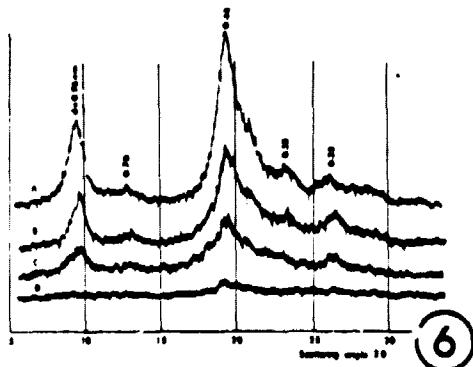


Figure 6 X-Ray Diffraction

A: standard chitin, B: prepared T. mentagrophytes cell wall acids, alkali-treated specimen, B: E. floccosum fraction of the same type,

C: prepared cell walls of both types

With regard to the presence of proteins with the above-mentioned features (peptides) in the dermatophyte cell walls, almost no knowledge is now available. The same is true of other true fungi. Analyses by peptide mapping of proteins in the cell walls of Aspergillus niger and Chaetomium globosum have been carried out, but nothing definite has been determined [67].

However, we have studied E. floccosum by isolation of the outer layer of a 3-layered cell walls. It has been discovered that this layer is mainly made from peptides (63%) [33]. When this fraction was reacted with Helicase (digestive enzyme of *Helix pomatia*), the 2 inner layers were easily dissolved. However, the outside layer was not decomposed. Figure 7 shows an isolated outer layer (exolayer) thin specimen image. It is clear that the structure is a film-like structure with a thickness of 200-500 Å. Figure 8 is an image by staining. A roughnet-like structure is seen. As was previously mentioned (Figure 5), freeze-fractured images are observed as a smooth structure. This structure is that of a glycopeptide containing only mannose. Furthermore, aspergillic acid and glutamic acid [66], which are thought to participate in bonding of cerine, seronin, and N-glycosyl, which seem to be the mannose or O-mannose bonds, occupy 41% of the entire amino acid and glycine and alanine, which are important component of peptide glycans of bacteria, occupy 27%. This fact appears to show that an

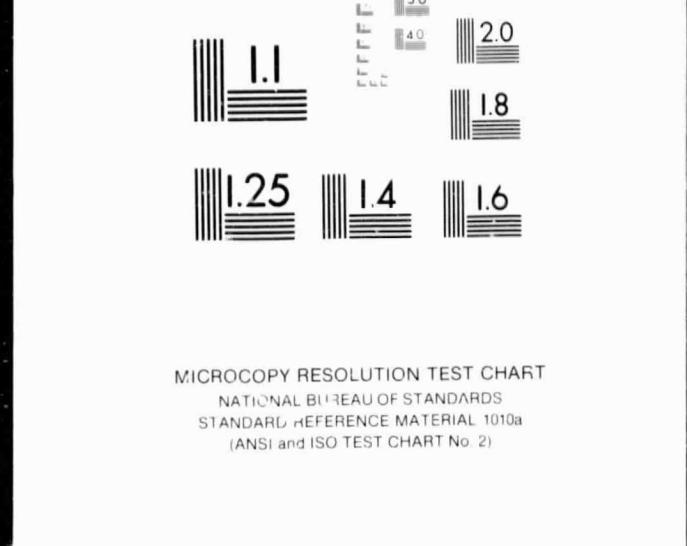
irregular peptide glycan-like layer is present in the outermost layer of the walls of cells of true fungi, as is the case with bacteria cell walls. The outermost layer of cell walls of T. mentagrophytes was recently isolated and it appears that the main component is protein (80%), while the walls also contain glucomannone (7%).

In any case, there are still many unclear points with regard to the protein content of cell walls of dermatophytes. Gander[69] has reported in detail on the glucoproteins of the cell walls of other true fungi.

3) Fat Components

The method of extraction of bound-lipids and easily extracted lipids by Bartnicki-Garcia [4,70] and the method of Bligh and Dyer [71] can be mentioned as methods for extraction of fats from the cell walls of true fungi. The bound lipids in the former method contain many other lipids and the latter is a useful method for analysis of fats. The amount of fat in dermatophyte cell walls is 2-7 % (Table 1).

However, only the fat composition of *E. floccosum* has been analyzed and reported and the presence of triclycerides and liberated fatty acids has been clarified[31]. Traces of sterols, lymph fats, etc. have been detected. However, these appear to be mixed with the membrane components. The presence of ribopolysaccharides, such as those seen with gram negative fungi, has not been confirmed.



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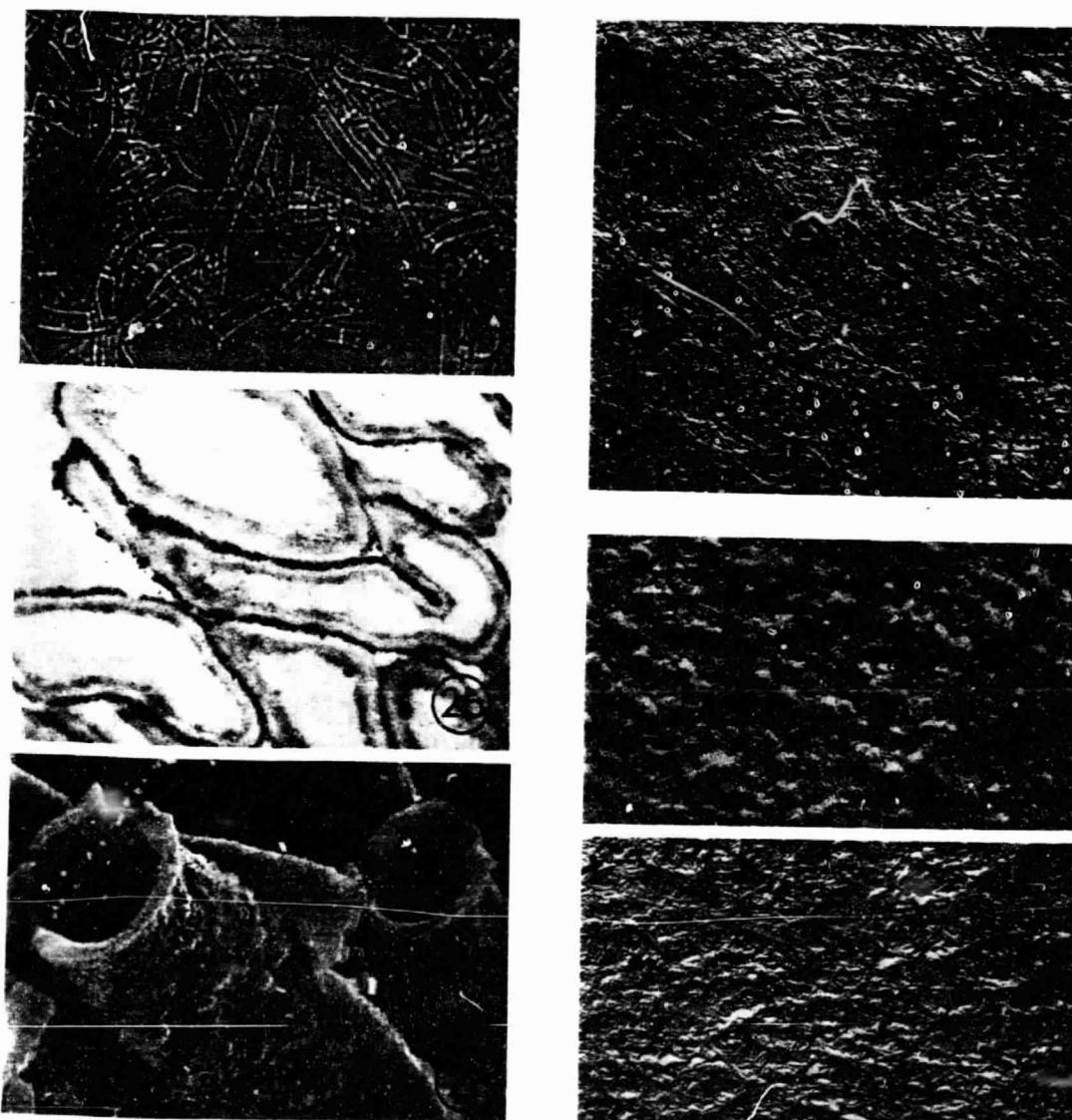
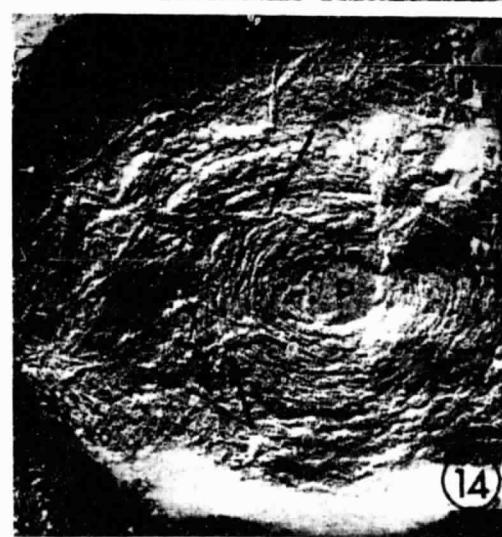
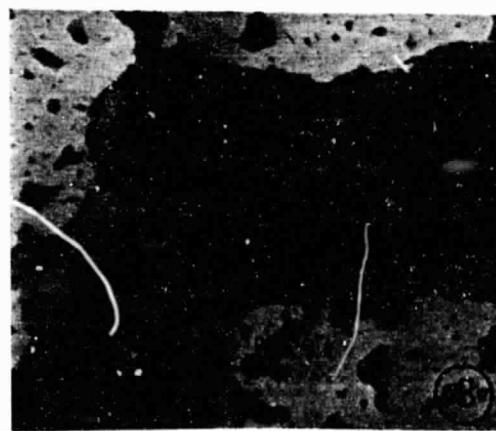
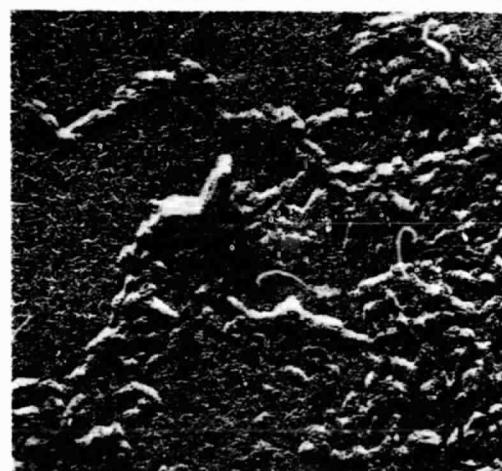


Figure 2a Phase Differential Micrograph of Cell Wall Fraction of E. floccosum X 400
 2b Same thin layer micrograph; 3-layered structure noted. x 11,000
 2c Same scanning electron micrograph; x 5,000

Figure 3 E. floccosum inner layer shadowing micrograph; fibrile and amorphous matrix structure, x 36,000

Figure 4a Shadowing micrograph of outermost layer of E. floccosum; fibrile structure not noted; x 36,000
 4b Shadowing micrograph of outermost layer of E. floccosum isolated by SDS treatment; different structure from inner layer; x 36,000



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Figure 5 Freeze fracture of E. floccosum; E: tear along outer layer of cell wall; C: cross section of cell wall; PM tear of cell membrane (surface A); CP: cell cross section, x 36,000

Figure 7 Outer layer (ex-layer) of walls of cells isolated from E. floccosum, x 36,000

Figure 8 Negative stained specimen of outer layer of E. floccosum, x 10,000

Figure 11 Shadowing micrograph of alkali-treated fraction of T. mentagrophytes; diameter of approximately 200 A, x 100,000

Figure 12 Shadowing micrograph of fraction treated with acid (Figure 12); Fraction with which chitin is identified in X-ray diffraction, x 100,000

Figure 14 septa of T. mentagrophytes; middle layer noted in electron transmission of thin slice (mainly chitin; microfibrile structure from inside of hyphae cell walls (arrows)), x 50,000

4. Structural Model of Cell Walls

1) Structure of Hypha Cell Walls

The micromorphology and chemical structure of cell walls was discussed in above-mentioned item 2. However, here we will discuss the structural models of cell walls based on this data.

The two methods of electron micrographic cell chemistry and chemical analysis of the soluble and residue portions of alkali and acid enzyme treatment of cell walls will be considered for determinations of the chemical properties of the cell structure of cell walls. Poulain showed a model for the cell walls of T. mentagrophytes with the former method in 1976 (Figure 9).

Researchers have used the latter method. However, only T. mentagrophytes [26, 30] and E. floccosum [27] have been used.

Furthermore, one method for fractionation of the cell walls of T. mentagrophytes is shown in Figure 10.

Moreover, the chemical structure of this fraction is shown in Tables 2, 3, and 4. First of all, fraction Sup-I that has been made soluble by initial alkali treatment has an amorphous matrix structure with fiber crevices. It is made from a polysaccharide whose main component is galactomannone having a 1,6 bond, 1,2 bond and 1,3 bond and a glucane whose main chain is a β -1, 6 and β -1, 3 bond. The residues that are obtained after these fractions have been extracted are made from only microfibriles, as shown in Figure 11. With regard to these microfibriles, the fibrile structure is lost by removal of the Sup-II of the acid-soluble

fraction. The structure changes to a crystal chitin structure, as shown in Figure 12. Consequently, the microfibriles appear to be made into fibriles by the glucomannone peptide complex (Sup-II) which is soluble in acids and nonsoluble in alkalis [26]. The same findings are obtained for E. Floccosum.

The model of the cell walls is shown in Figure 13 in order to clarify the above-mentioned points. Of the 3-layered structure observed by observation of thin slices with electron microscopes, the outermost layer (exolayer) is made mainly from a sugar-protein layer whose main component is protein and which is 200-500 Å thick. The 0.1-0.2 middle layer is a dense layer of microfibriles made mainly from chitin and glucane. The inner layer is 0.1-0.2 thick and has a rough microfibrile structure. Amorphous galactomannone-glucane-peptide complexes are found in the crevices.

2) Septum Structure:

The septa that were observed by microscopic analysis of thin specimens were made from 3 layers, of which one layer in between two other layers had a low electron density [26, 56]. This structure is the same structure as that of true fungi, including dermatophytes. In the case of T. mentagrophytes, the surface that makes contact with the cell membrane has the same fibrile structure as the inner layer of the cell walls of hypha [26]. However, this layer is peeled off by papain treatment and presents a structure where the microfibriles in the middle layer are arranged concentrically (Figure 14). This microfibrile structure appears to be made mainly of chitin [26]. Figure 15 is a model of this structure [26].

Table 2 Sugar Composition of Each Sup Fraction of T. mentagrophytes Cell Walls 30

	a 細胞壁	Sup-I	Sup-II	Sup-III
b 糖含量 (%)	47.9	67.5	58.0	47.0
c 構成糖比	man:glc 1:3.1	man:glc:gal 7.9:1.1:1	man:glc 1:1.4	glc 1
d ペプチド含量 (%)	7.8	7.5	23.8	0

man: mannose, glc: glucose, gal: galactose

Key: a. cell walls b. sugar content (%) c. structural sugar ratio
d. peptide content (%)

Table 3 Sugar Composition of Each Res Fraction of T. mentagrophytes
Cell Walls 30

	a 細胞壁	Res- I	Res- II	Res- III
b 糖 含 量 (%)	47.9	22.9	12.9	3.3
c 糖 成 糖 比	man : glc 1 : 3.1	man : glc 1 : 5.3	glc 1	glc 1
d N.アセチルグルコサミン (%)	37.4	50.4	62.6	77.1

man: mannose, glc: glucose

Key: a. cell walls b. sugar content (%) c. structure sugar ratio
d. N-acetylglucosamine (%)

Figure 4 Sugar Composition of Each Pt Fraction of T. mentagrophytes
Cell Walls

	Sup- I	Pt- I	Pt- II	Sup- I'
a 糖 含 量 (%)	67.5	83.9	68.8	23.9
b 糖 成 糖 比	man : gal : glc 7.9 : 1.1 : 1	man : gal 25.7 : 1	man : gal : glc 16.4 : 1 : 2.8	man : glc 1 : 1.3
c ペプチド含量 (%)	7.5	12.5	6.8	0

man: mannose, glc: glucose, gal: galactose

Key: a. sugar content (%) b. structure sugar ratio c. peptide content (%)

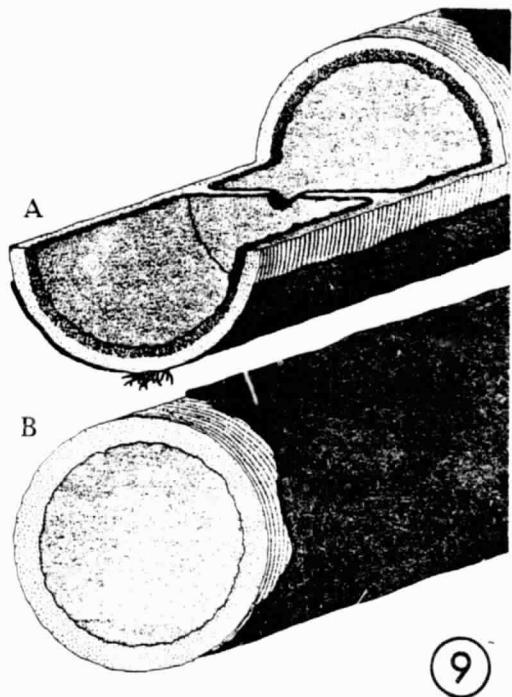


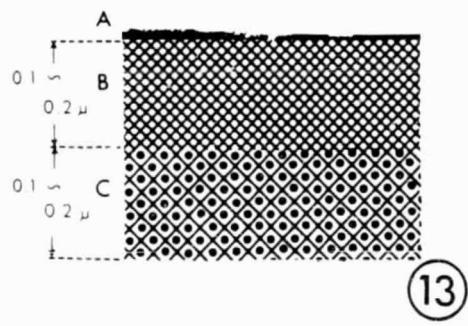
Figure 9 Model of Cell Walls of *T. mentagrophytes* Observed by
Electron Microscopic Cell Chemical Staining [25]

A: 3 layered structure of hypha cell walls, B: inner layer lost in mature hypha. The inner layer is mainly composed of galactomannane and the middle layer is composed mainly of chitin. The outer layer is a layer with a microfibrile structure.



Figure 10 Fractionation of Polysaccharides in Cell Walls of T. mentagrophytes [30]

Key: a. prepared cell walls b. centrifugation for 5 hours in 1 N NaOH at 100°C
 c. supernatant fluid d. cold ethanol
 e. sedimentation f. felling liquid
 g. dialysis h. ethanol chloride
 i. freeze-drying



(13)

Figure 13 Cell Wall Models for T. mentagrophytes and E. floccosum

A: outer layer (exolayer) made mainly from sugar proteins, B: layers made mainly from chitin-blucan fibriles, C: layer made from fibriles in B and polysaccharide proteins soluble in an alkali

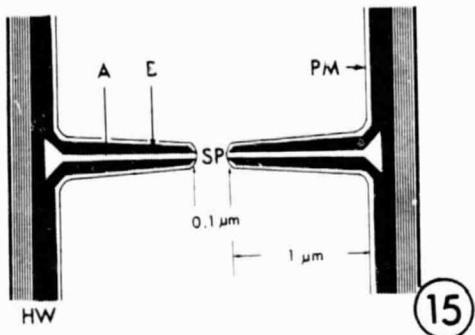


Figure 15 Cell Wall Model for T. mentagrophytes

A: chitin layer, B: layer made connected to inner layer of cell wall, made from microfibriles and amorphous matrix, PM: cellular membrane, HW: hypha wall

5. Morphology of Cell Walls

The following method will be considered for the synthesis of polysaccharide protein complexes, which are the basic microfibrile and matrix substances arising from chitin, which is found in cell walls. That is, 1. oligosufats synthesized from rribosomes [72] or membrane-like structures[73] and small amounts of peptides are first excreted to outside the cell membrane and self-assembly then occurs [74-76]. 2. polysacchride-synthesizing enzymes, such as chitin-synthesizing enzymes, are excreted to outside the cell or to the cell membrane[77-79]. Synthesis therefore occurs outside the cell in this method.

In contrast to the fact that a yeast protoplast is not noted in cell cultures and the formation of cell wall microfibriles is not seen, precursors that are excreted to outside the cell are seen in gel cultures and therefore, self-assembly is possible. Necas has studied this theory in detail [74]. Furthermore, there are other mentions of polysaccharide synthesizing enzymes in the bibliography.

The former method involves an N-acetylgluocamine -GDP and chitin-synthesizing enzyme. When these are incubated, chitin fibers are made from the enzyme particles [80]. That is, synthesis of

chitin particles occurs. Furthermore, there have recently been reports that the chitin-synthesizing enzyme chitinogen is present in the cell structure of the yeasts [79]. On the other hand, observations of freeze fractures have resulted in discovering that the synthesis of cellulose microfibriles in plant cells occurs from particles that appear to be enzymes on the cell membrane [81-83].

Nevertheless, in dermatophytes there is little information on chitin-synthesizing enzymes and polysaccharide-synthesizing enzymes, in contrast to the large amount of information of the above-mentioned yeasts. The two above-mentioned hypothesis should be used if it were concluded that the cell walls of dermatophytes could also have the same structure as that of yeast-type true fungi cell walls.

Conclusion

As was previously mentioned, the presence of a microfibrile structure whose main component is chitin and a sugar protein complex that is soluble in an alkali (amorphous matrix) was confirmed in studies on the cell walls of dermatophytes. However, the molecular structure of the sugars and the bonding mechanism with proteins (peptides) was not clarified. This will be studied in the future. It will be important in solving the many problems of bacteriology.

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